Chapter

Transmission Electron Tomography: Intracellular Insight for the Future of Medicine

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Abstract

Transmission electron microscopy (TEM) gives a good image for ultra-intracellular organelles in two-dimensional projections, but to get a three-dimensional structural information, we should use the high-voltage transmission electron tomography (TET). The use of TET is important to resolve the questions about the relation between the different cell organelles and their mechanisms of action to correlate structure to function for medical treatment solutions.

Keywords: transmission electron tomography, organelles, three-dimension, cells, histologists

1. Introduction

Transmission electron microscopy (TEM) had been largely responsible for shaping our views of organelle architecture, as it could provide the highest resolution within a spectrum of complementary tools used in the structural study of organelles in biological specimens. The images are two-dimensional projections, which pass through a relatively small slice of the specimen, and features from different levels are superimposed. This has enabled histologists in many circumstances to regard the third dimension as constant and interpret the image accordingly. In conventional thin-section TEM, the sections were generally much thinner than the specimen, and overlap of features was a problem. When the high voltage electron microscopes were used, the specimen with thick section-cut could be better examined. But the overlapped details of these images in two-dimensional images were more difficult to be interpreted. Transmission electron tomography (TET) resolved many of the limitations observed with serial thin section reconstruction by using sections thick enough (from 200 to 2000 nm) to contain a significant fraction of the organelle within the section volume allowing computing three-dimensional (3D) reconstructions of objects from their projections recorded at several angles by the use of high voltage (400–1000 kV) TEM [1].

Specimens were incrementally tilted in ET by a range up to ±60°, and many images were taken at each tilt. So, these serial images represented the whole specimen from different views. These serial images were aligned and then re-projected to give a 3D reconstruction or what is called a tomogram of the specimen. Therefore, the electron tomography represented the most available technique with high resolution to examine the biological specimens as cells [2, 3].

2. Specimen preparation

The most common methods for preserving cell structure for TEM and TET were based on chemical fixation using glutaraldehyde for primary fixation and osmium tetroxide for secondary fixation. Following fixation, water was replaced by an organic solvent that was then replaced with a resin (**Figure 1**) [4].

Frozen-hydrated specimen was another method of preparation as a hydrated specimen was frozen rapidly enough, and then the liquid water was transformed into a vitreous solid state with a structure and density similar to those of the liquid. Sections of frozen-hydrated specimens were done using cryo-ultramicrotome and then examined by ET using a special holder. Cryo-sectioning combined the advantages of rapid freezing with the use of bulk material, and it was applicable to whole cells and tissues [1, 5].

Cryo-ET could directly image thin regions of cells that were adherent or grown on EM grids. So, it was becoming the method of choice for providing 3D information about intact intracellular structures at molecular resolution [6].

The plastic sections formed from resin cuts had more contrast because these sections were stained by heavy metal stains. Therefore, the images were been more close to focus. On contrast to the frozen-hydrated specimens, they had less contrast and should be imaged using a large defocus to get maximum phase of contrast [3].

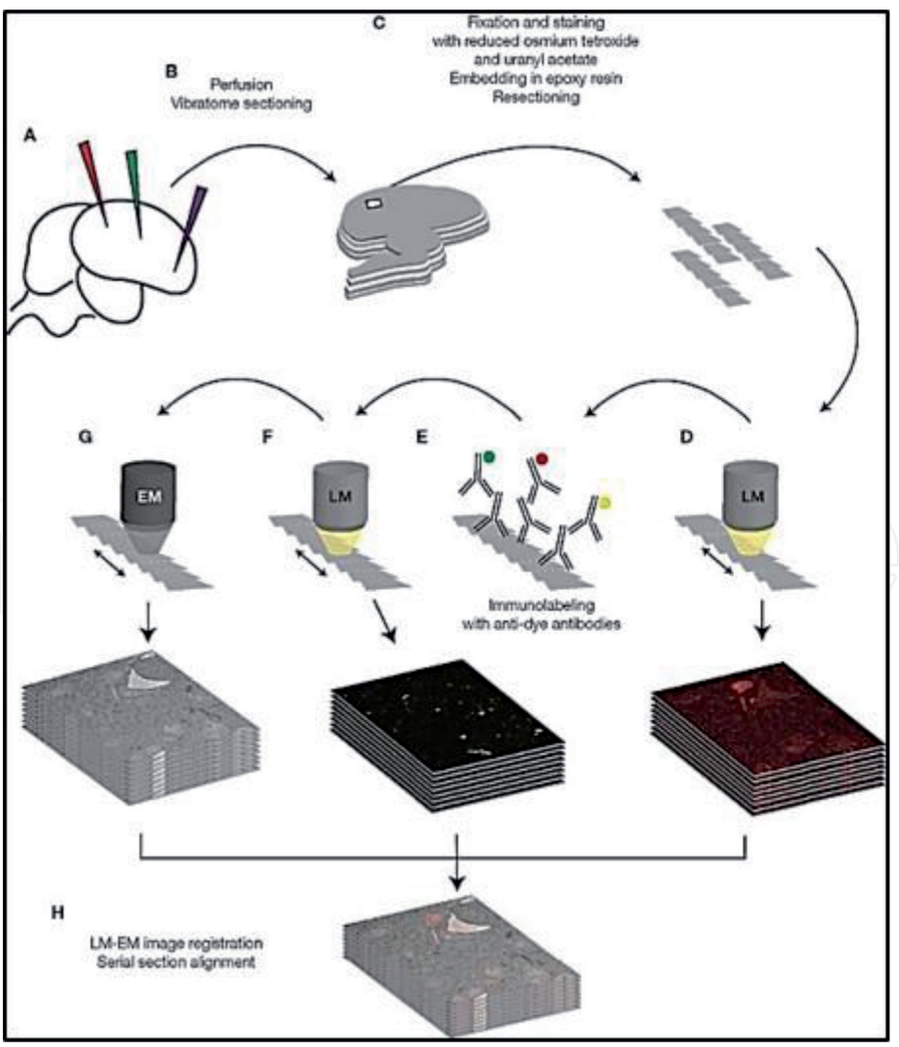

Figure 1.
Chemical fixation and plastic embedding [quoted from reference 5, available from: PubMed Central License: CC BY 4.0].

Sample thickness was generally limited to about 200 nm in 100 kV instruments and approximately twice than that in 300 kV instruments. While higher voltage EMs existed, these instruments were extremely rare and only moderately expanded (approximately another doubling) the permissible specimen thickness [3].

3. Reconstruction technique

Following image acquisition, image processing was divided into three phases. The first two phases were generally termed alignment and the last phase was the three-dimensional reconstruction proper. The images with tilt series should be processed to rotation and stretch to be aligned, and this was an important step to compensate any minimum difference in magnification, image rotation, and translation. The tilt axis of each image also was determined before the three-dimensional reconstruction [3]. Many software programs were used to perform these reconstructions as IMOD [7], TOM Toolbox [8], and SPIDER [9].

4. Examples of cellular organelles examined by TET to resolve questions about their mechanisms of action

4.1 Mitochondria

Mitochondria were among the first intracellular organelles to be studied extensively due to their importance in energy metabolism. Most observed mitochondria had large volume of matrix forming a compartment lined by inner membrane called the inner boundary membrane pushed against the outer membrane. A narrow space was seen between these two membranes of about 8 nm wide. The inner membrane projected into the matrix at discrete loci called crista junctions which were of a uniform diameter. The shape of cristae and the number of crista junctions were variable and dynamic in nature according to site of mitochondria and state of function. Electron tomography was also a valuable tool in showing the possible mechanisms of apoptosis as cytochrome c release was not effected by mitochondrial swelling and rupture of the outer membrane but apparently through the formation of large pores in the outer membrane through which intact cytochrome c could pass [1].

Using transmission electron tomography, investigators mapped the 3D topologies of some membrane organelles in the mouse ventricular myocardium, including transverse tubules (T-tubules), junctional sarcoplasmic reticulum (SR), and mitochondria. This research illustrated the geometric complexity of T-tubules. Electron-dense structures were usually seen between the outer membrane of the mitochondria and SR or T-tubules. Some investigators proposed that the relation between the mitochondria and the nearby structures are so important to local control of calcium in the heart, including the establishment of the quantal nature of SR calcium release, which is known as calcium sparks [10].

4.2 Endoplasmic reticulum (ER)

One of the benefits of using electron microscope tomography and live-cell imaging was to determine the mitotic assembly of the nuclear envelope, which was shown to be primarily originating from endoplasmic reticulum (ER) cisternae. Also, the nuclear pore complexes assembly occurred after the completely formed nuclear envelope. The chromatin-associated Nup107–160 complexes were in single units instead of assembled pre-pores. Therefore, the investigators proposed that

the post-mitotic nuclear envelope assembled directly from ER cisternae which were followed by membrane-dependent formation of nuclear pore complexes [11].

4.3 Golgi apparatus

As the Golgi apparatus is so big and a single tomogram cannot capture much of its organization, "montage" tomograms were acquired from serial sections and then merged both laterally and "vertically" *in silico* to reconstruct a 4 mm^3 cellular volume. The 3D model of this merged serial tomogram illustrated the complexity of the true organization of the Golgi body and also led to the discovery of vesicle-filled "wells," which are formed by the aligned fenestrae from a series of cisternae [12].The inter-cisternal connections might function in retrograde and possibly anterograde transport of Golgi enzyme and lipids but not in transport of maturated components [13].

4.4 Desmosomes in cell junctions

A human skin biopsy sample was high-pressure-frozen, cryo-sectioned, and imaged by electron cryo-tomography to examine the desmosomes which are cadherin-mediated intercellular junctions. They are important to support the cell junction for tissue reinforce. The cryo-tomograms revealed that the cadherin molecules were densely and uniformly packed. They were at first arranged as small interacting groups with extracellular domains to form cis-homodimers then the opposing cell membrane approximated to form trans-homodimers. After the initial formation was established, more molecules were linked to the contact zone and the junction became more compacting. This process was regularized by building blocks of alternate cis and trans dimers, and the strength of cell to cell contact became homogeneous. These processes were repeated to have finally a fully mature desmosome [14].

4.5 Actin filaments

By the use of cryo-ET, some investigators revealed that the membrane cytoskeleton consisted of actin filaments mainly and the other associated proteins. This membrane cytoskeleton covered the whole cytoplasmic surface. Moreover, it was closely related to clathrin-coated pits and caveolae. The actin filaments which were linked to the cytoplasmic surface of the plasma membrane were likely to form the boundaries of the membrane compartments responsible for the temporary confinement of membrane molecules, thus partitioning the plasma membrane with regard to their lateral diffusion [15].

4.6 Microtubules, cilia, and flagella

The axoneme forms the essential and conserved core of cilia and flagella. Cryo-electron tomography was used to examine Chlamydomonas and sea urchin flagella to know information about the composition of axonemal doublet microtubules (DMs). These studies showed that B tubules of DMs contained 10 protofilaments (PFs) and also that the inner junction as well as the outer junction between the A and B tubules are different. The outer junction, important for the initiation of doublet formation, was formed by close interactions between the tubulin subunits of three PFs with unusual tubulin interfaces. The inner junction was formed of an axially periodic structure connecting tubulin PFs of the A and B tubules. The discovered microtubule inner proteins (MIPs) on the inside of the A and B tubules were observed to be more complex than previously thought, as they are composed

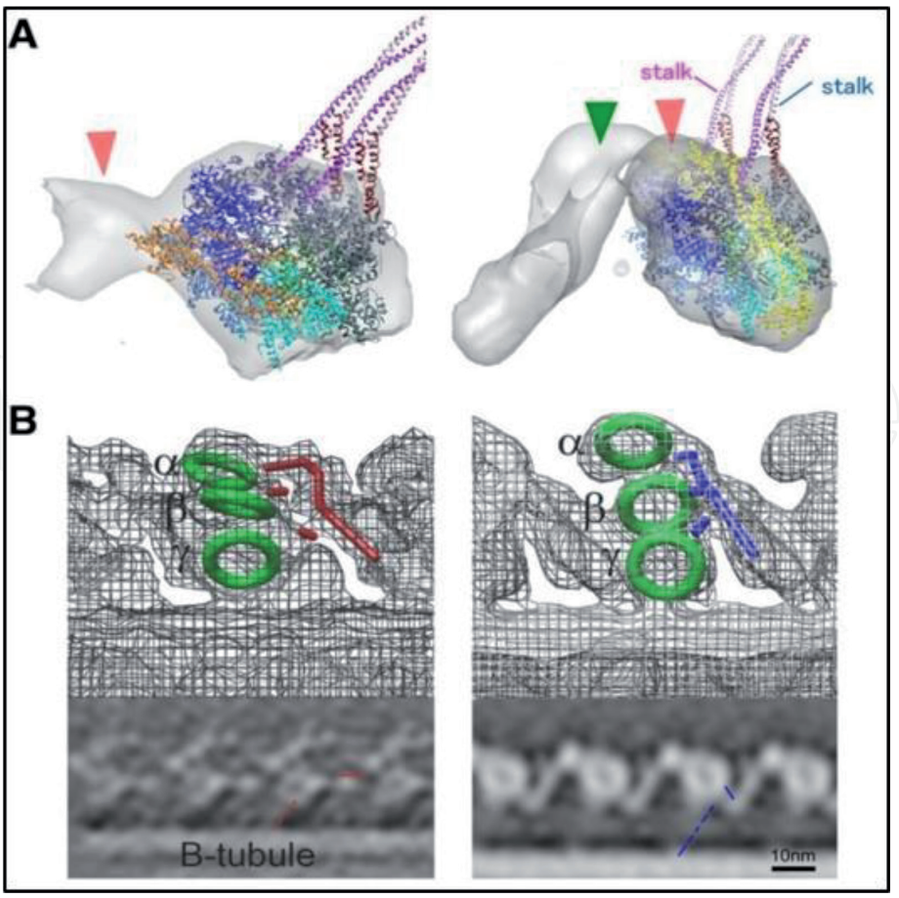

Figure 2.
Structural change of dynein induced by nucleotides: Left: structure of pre-power stroke; Right: structure without additional nucleotide (post-power stroke). (A) Tomography structure of mouse respiratory cilia consisting of two dyneins, the linker is shown in orange in pre-power stroke form and yellow in post-power stroke forms. (B) Tomography structure of Chlamydomonas, showing shift of the head as green rings and orientations of the stalk as blue and red dotted lines, as well as the neck domains and N-terminal tails (as red and blue solid lines) [quoted from reference 17, License: CC BY 4.0].

of alternating small and large subunits with periodicities of 16 and/or 48 nm. MIP3 formed arches connecting B tubule PFs. Also, the "beak" structures within the B tubules of the investigated Chlamydomonas DMT1, DMT5, and DMT6 were seen to be composed of a longitudinal repeating band of proteins with a periodicity of 16 nm [16].

The 3D structural analysis from cryo-EM has been playing indispensable role in motor protein research as a potential method to analyze 3D structure of complexes of motor and cytoskeletal proteins. The 3D image classification proved nucleotide-induced conformational change of dyneins and interesting distributions of multiple forms of dynein in the presence of nucleotides in cilia (**Figure 2**) [17].

Some authors reported that the centrosome-associated microtubule (MT) ends could be closed or open. The closed MT ends were more numerous and were distributed in a uniform matter around the centrosome. On the other hand, the open ends were found on kinetochore-attached MTs. These results showed the structural participations for models of microtubules interactions with centrosomes [18].

5. Telocyte; the entire cell examined by TET

A telocyte is a special type of interstitial cell having prolongations named telopodes [19, 20]. This cell has been described by electron microscope in different organs and tissues in the body. Some investigators examined the heart

ultrastructure by TET and mentioned that telocytes make a network in the myocardial interstitium, which is involved in the long-distance intercellular signaling coordination. The cardiac telocyte network could integrate the overall "information" from vascular system (endothelial cells and pericytes), nervous system (Schwann cells), immune system (macrophages and mast cells), interstitium (fibroblasts and extracellular matrix), stem cells/progenitors, and working cardiomyocytes [21].

Other authors suggested that the telocytes present in the lamina propria of rat jejunum could be a heterogeneous population having different members which could switch between one activation states to another. They had cell to cell communication by paracrine mechanisms and to act as stem cell adjutants involved in epithelium renewal [22].

Transmission electron tomography has revealed complex junctional structures and tight junctions connecting pleural telocyte and small vesicles at this level in telopodes. Thus, pleural telocytes share significant similarities with telocytes described in other serosae. The extremely long thin telopodes and complex junctional structures that they form and the release of vesicles indicate the participation of telocytes in long-distance homo- or hetero-cellular communication [23].

6. Synapses in special sense organs

The cochlear inner hair cells (IHCs) are highly specialized cells in continuous stimulation and with rapid turn-over membrane. These cells synapse with multiple afferent nerve fibers. The ability of the IHC synapse to sustain activity for long periods is due to the presence of the synaptic ribbon. The use of 3D reconstruction by TET of an IHC infra-nuclear region revealed a network of rough endoplasmic reticulum (rER), mitochondria, and vesicles related to the synaptic ribbon (**Figure 3**). Small vesicles (about 36 nm) were seen by TET tethering to the rER. These vesicles were not observed either budding or fusing with the rER membrane, but only

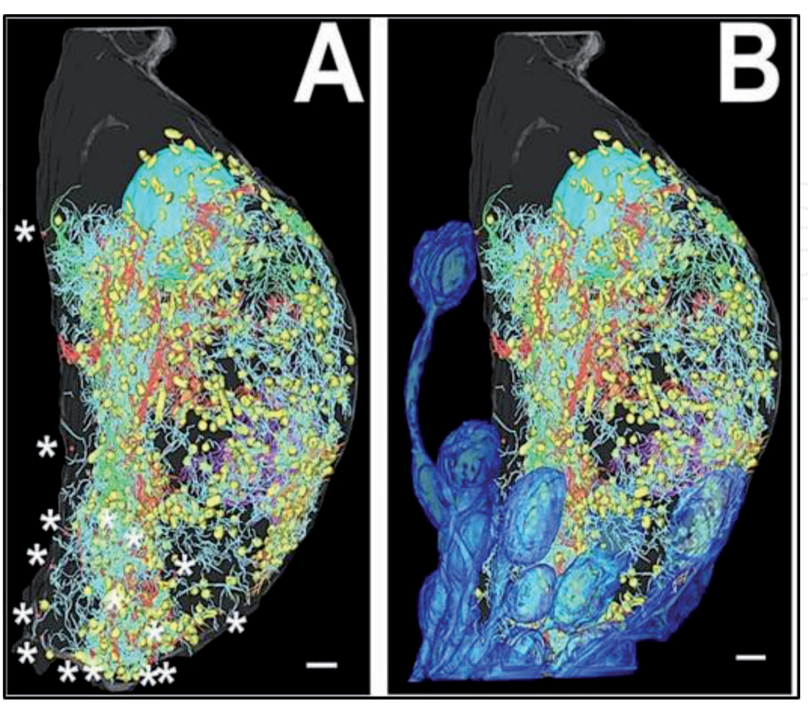

Figure 3.
Complete model of membranes, mitochondria, and synapse distribution in cell. (A) Ribbon synapses (red spheres) are marked with asterisks for clarity. (B) Reconstructions of synaptic terminals are shown (blue). [Quoted from reference 24, License: CC BY 3.0].

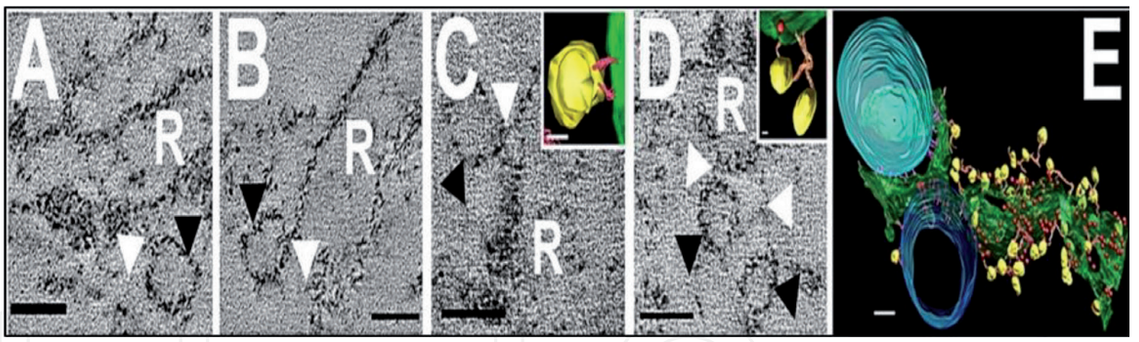

Figure 4.
TEM and TET reconstruction of vesicles link on rER and at the ribbon synapse. (A–D) Linkages (white arrowheads) are shown between membrane vesicles (black arrowheads) and three different areas of the rER (R). Insets in C and D show reconstruction of these links. (E) Reconstruction of a section of rER (green with ribosomes in red) with linkages to mitochondria (blue) and vesicles (yellow) showing the linkages surrounding the membrane. [Quoted from reference 24, License: CC BY 3.0].

connected to it by filamentous linkages. This result showed the possibility that this membrane network might represent a secondary store of neurotransmitter vesicles to be released during sustained synaptic transmission (**Figure 4**) [24].

7. Conclusion

Transmission electron tomography is not only an important instrument in the deep insight of the cellular components needed by histologists but also give information about mechanistic hypotheses, which may help scientists to correlate structure and function to different ways of getting diseased and to know the ways of medical treatment. It also helps to understand the disturbances of these organelles that cause the progression of damage in going more aged.

The integration between different basic sciences such as histology, pathology, physiology, bacteriology, and pharmacology, and the clinical physicians can be achieved through group of researches to cover some unclear points about different biological structures for the future of medicine.

Conflict of Interest

No conflicts declared.

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